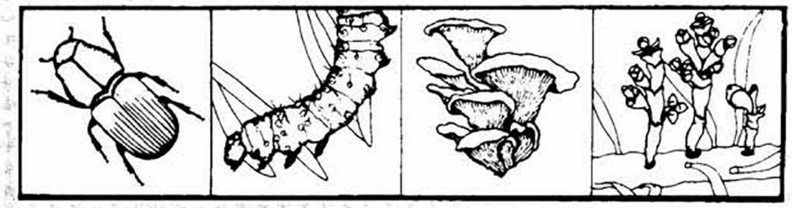
Insect and Disease Management



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ROOT DISEASES OF WESTERN WHITE PINE TRANSPLANTS - USDA FOREST SERVICE NURSERY, COEUR D'ALENE, IDAHO

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ABSTRACT

Decline and mortality of western white pine transplants at the USDA Forest Service Nursery in Coeur d'Alene, Idaho were investigated. Transplants were container-grown seedlings further grown in bareroot beds for one (plug+1) or two (plug+2) growing seasons. Chlorosis and necrosis were common on many transplants. Often, transplants with these symptoms were in groups. About 19 percent of the examined plug+1 transplants were chlorotic, whereas necrotic transplants comprised about 11 percent of the examined plug+2 transplants. Transplant height was adversely affected in heavily diseased plug+2 beds. Several species of *Cylindrocarpon*, *Fusarium*, and *Pythium* were frequently isolated from roots of healthy, declining, and dead transplants. Several of these fungi were likely carried on roots of container-grown seedlings and proliferated when seedlings were transplanted into fumigated soil.

INTRODUCTION

Western white pine (*Pinus monticola* Dougl.) is an important conifer species in the Inland Northwest. Recent efforts to improve resistance of this species to devastating white pine blister rust, caused by the fungus *Cronartium ribicola* Fisch., have resulted in seed orchards producing high value seed. Forest managers usually desire as many blister rust resistant white pine seedlings for outplanting as can be produced at area nurseries. Sometimes they require seedlings with larger caliper and root systems than can be produced in containers in one year or two years in bareroot operations. To provide seedlings best suited for special outplanting sites, growers at the USDA Forest Service Nursery in Coeur d'Alene, Idaho have recently experimented with transplanting either 1+0 container or 2+0 bareroot stock for either one or two years to improve caliper and root systems. Such transplant operations are expensive and result in much higher per seedling costs. However, growers hope high costs can be offset by higher survival and faster intial growth on adverse outplanting sites.

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Transplanted seedlings are typically grown in fields fumigated with dazomet (Basamid®) prior to transplanting. The primary goal of such fumigation is to reduce or eliminate soilborne populations of potential pathogenic organisms, primarily members of the fungal genera *Fusarium* and *Pythium*. If done properly when soil temperatures and moisture content are right, dazomet fumigation is usually quite effective in killing these and other soil-borne organisms (James and others 1990).

During one of the recent transplant crops at the nursery, extensive mortality was noticed in the fall of the second growing season where container transplants (plug+2) were being grown in bareroot beds (Figure 1). This mortality was most apparent in portions of Field 2. Although some affected transplants were scattered throughout beds, there were also definite mortality centers (Figure 1). Mortality was not limited to plug+2 transplants, but was also common in container transplants grown the first year after transplanting (plug+1). Many transplants exhibited declining vigor and appeared chlorotic (Figure 2). Necrotic transplants had red, brown or black foliage. Similar damage was noticed before at the nursery (James and Gilligan 1986).

Growers were concerned about the extent of decline and mortality of transplants. They wanted to know about possible associations of pathogenic root-infecting fungi with the problem. Therefore, an evaluation was conducted to determine the extent of transplant decline and mortality and quantify occurrence of potentially pathogenic fungi on transplant roots.

MATERIALS AND METHODS

To determine the extent of transplant decline and mortality, temporary plots were established in various portions of transplant beds to determine percentage of plants with symptoms. In plug+2 beds, plots 1 foot in length were established across beds of transplants in the northern portion of Field 2. Eight beds were numbered linearly from south to north. The first plot was established 100 feet east of a north-south road; five plots were established per bed at 10 feet intervals in an easterly direction. Five rows of transplants were present in beds. Within each plot, the number of healthy (completely green foliage), chlorotic (mostly yellow foliage) and necrotic (mostly red, brown or black foliage) transplants were counted. In addition, heights of five randomly selected healthy transplants in the center of each plot were measured from the groundline to the tip of the terminal bud. In plug+1 beds, plots 10 feet in length were established across beds of transplants located in the southern portion of Field 2. Four beds were numbered linearly from south to north. The first plot was established 20 feet from the same north-south road; three plots were established per bed with 10 feet between plots in an easterly direction. Five rows of transplants were present in beds. Within each plot, the number of healthy, chlorotic, and necrotic transplants were counted.

To quantify potentially pathogenic fungi on roots of transplants, several were selected for root isolation work. In plug+2 beds, 22 healthy (without disease symptoms), and 25 chlorotic transplants were randomly sampled. In plug+1 beds, 50 healthy and 20 chlorotic transplants were selected. Selected transplants were carefully excavated to retain most of their root system which was carefully washed to remove most adhering soil particles. Twenty root tips per transplant were randomly selected, excised to a length of about 2-3 cm, surface sterilized 1 minute in a 10 percent bleach solution (0.525 aqueous sodium hypochlorite), and rinsed with sterile water. Ten root tips from each transplant were placed on each of two selective agar media for enumerating presence of potentially pathogenic fungi. One medium was selective for *Fusarium* and closely related fungi (Komada 1975). The other medium was selective for *Pythium*, *Phytophthora*, and associated water mold fungi. This latter medium consisted of V-8 juice agar amended with several antibiotics. Plates of Komada's media were incubated for 7-10 days under diurnal cycles of cool, fluorescent light and plates of V-8 juice agar were incubated in the dark for 3 days. Fungi emerging from root tips were initially identified on selective media. Selected isolates were transferred to potato dextrose agar (PDA) and carnation leaf agar (Fisher and others 1982) to confirm identification. Percentage of transplants and collated root tips infected with selected fungi were calculated.

Figure 1. Plug+2 western white pine transplant mortality at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. Note concentrations of mortality.

Figure 2. Chlorotic plug+2 western white pine transplant (center) surrounded with healthy (non-symptomatic) transplants at the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

RESULTS AND DISCUSSION

Level of root disease symptom production was high in both plug+1 and plug+2 transplants (Tables 1 and 2). Higher levels of chlorotic transplants were found in plug+1 transplants, whereas more older transplants were dead. Although height of plug+2 transplants varied widely among different beds sampled (Table 2), the shortest transplants were often found in two beds with the greatest disease levels (beds 7 and 8).

Table 1. Incidence of root disease symptoms on plug+1 western white pine at the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

	Percent of Examined Transplants[1]			
Bed	Healthy	Chlorotic	Necrotic	No. Transplants Examined
1	97.6	1.7	0.7	697
2	67.1	32.7	0.2	438
3	64.8	34.4	0.8	660
4	90.8	8.8	0.4	456
All Beds	80.7	18.7	0.6	2,251

[1] Transplants examined for foliar indications of root disease: healthy transplants had dark green foliage throughout most of their crown; chlorotic transplants had yellow foliage throughout at least half their crown; necrotic transplants had either red, brown of black foliage throughout most of their crown.

Table 2. Root disease symptoms on plug+2 western white pine transplants at the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

	Percent of Examined Transplants[1]				
Bed	Healthy	Chlorotic	Necrotic	Avg. Height Healthy Transplants (mm)	No Transplants Examined
1	84.1	1.4	14.5	168[2]	69
2	97.5	0	2.5	179	81
3	98.8	0	1.2	210	83
4	97.1	0	2.9	181	70
5	89.3	1.2	9.5	127	84
6	93.8	3.1	3.1	122	65
7	77.3	1.0	21.7	118	97
8	76.5	1.0	22.5	122	102
All Beds	88.5	0.9	10.6	153	650

[1] Transplants examined for foliar symptoms of root disease: healthy transplants had dark green foliage throught most of their crown; chlorotic transplants had yellow foliage throughout at least half their crown; necrotic transplants had either red, brown, or black foliage throughout most of their crown.

Many different potentially pathogenic fungi were isolated from roots of both transplant types (Tables 3 and 4). By far, the most frequently isolated fungi were from the genus *Cylindrocarpon*. All sampled transplants, both healthy and chlorotic, were infected at some level with these fungi. *Cylindrocarpon destructans* (Zins.)Scholten was the most commonly isolated fungal species; encountered at slightly higher levels on chlorotic transplants, *C. destructans* colonized roots of every healthy (those without root disease symptoms) transplant sampled. Three other *Cylindrocarpon* spp. were less frequently isolated from either healthy or chlorotic transplants: *C. tenue* Bugn., *C. didymum* (Hartig) Wollenw., and *C. gracile* Bugn. Previous work with container-grown western white pine seedlings (James 1992; James and Gilligan 1990; James and others 1994) has shown that *Cylindrocarpon* spp. are very common on roots of both diseased and non-diseased seedlings. These organisms cause root decay of container-grown seedlings, often without production of above-ground disease symptoms (James and others 1994). *Cylindrocarpon* spp. are often seed-borne on five-needle pines (James 1989b, 1991), but generally do not cause high levels of damping-off. Likewise, little root disease occurs on nursery-grown seedlings (James and Gilligan 1990; James and others 1994). It is likely than many container-grown white pine seedlings are infected with *Cylindrocarpon* by the end of the greenhouse production cycle even though disease symptoms are unnoticeable (James and others 1994). Current investigations are underway to determine roles of *Cylindrocarpon* root infection on survival and performance of outplanted western white pine in selected forest environments.

Table 3. Root infection and colonization of plug+2 western white pine transplants by *Fusarium*, *Cylindrocarpon*, and *Pythium* spp., USDA Forest Service Nursery, Coeur d'Alene, Idaho.

	Healthy Transplants[1]		Chlorotic Transplants[2]	
Fungal Species	% Transplant Infection	% Root Colonization[3]	% Transplant Infection	% Root Colonization[3]
Fusarium				
proliferatum	59.1	13.6	88.0	31.2
oxysporum	81.8	24.5	60.0	10.0
acuminatum	72.7	13.6	36.0	6.4
sambucinum	13.6	1.8	8.0	0.4
solani	0	0	32.0	0.8
All *Fusarium*	95.4	47.7	92.0	47.7
Cylindrocarpon				
destructans	100.0	64.5	100.0	81.6
tenue	40.9	7.3	28.0	3.2
didymum	13.6	1.8	0	0
gracile	4.5	0.4	0	0
All *Cylindrocarpon*	100.0	74.5	100.0	84.8
Pythium				
ultimum	13.6	3.2	84.0	19.2
aphanidermatum	0	0	24.0	2.4
All *Pythium*	13.6	3.2	84.0	19.6

[1] Twenty-two transplants sampled.
[2] Twenty-five transplants sampled.
[3] Percent of root pieces (10 sampled per transplant) colonized with the particular fungus.

Table 4. Root colonization of plug+1 western white pine transplants by *Fusarium*, *Cylindrocarpon*, and *Pythium* spp. - USDA Forest Service Nursery, Coeur d'Alene, Idaho.

	Healthy Transplants[1]		Chlorotic Transplants[2]	
Fungal Species	% Transplant Infection	% Root Colonization[3]	% Transplant Infection	% Root Colonization[3]
Fusarium				
proliferatum	74.0	19.2	75.0	13.5
oxysporum	36.0	4.6	30.0	5.0
acuminatum	14.0	1.4	0	0
sambucinum	6.0	0.6	0	0
solani	0	0	10.0	1.5
sporotrichioides	0	0	20.0	2.5
equiseti	0	0	10.0	1.0
All *Fusarium*	86.0	25.8	95.0	20.5
Cylindrocarpon				
destructans	100.0	79.6	100.0	80.0
tenue	42.0	6.0	25.0	6.5
didymum	6.0	0.8	5.0	0.5
All *Cylindrocarpon*	100.0	85.2	100.0	85.5
Pythium				
ultimum	10.0	1.6	75.0	13.0
aphanidermatum	10.0	1.4	40.0	4.0
All *Pythium*	16.0	3.0	80.0	17.0

[1] Fifty transplants sampled.
[2] Twenty transplants sampled.
[3] Percent of root pieces (10 sampled per transplant) colonized with the appropriate fungus.

Several *Fusarium* species were also isolated from either healthy or chlorotic transplants (Tables 3 and 4). Overall levels of *Fusarium* root infection were similar between healthy and chlorotic transplants. The most commonly isolated species from plug+1 transplants was *F. proliferatum* (Matsushima) Nirenberg; this species was less common on older plug+2 transplants. The other common species was *F. oxysporum* Schlecht., which increased in prevalence the longer transplants were grown in bareroot fields. Other *Fusarium* spp. less frequently isolated included *F. acuminatum* Ell. & Ev., *F. sambucinum* Fuckel, *F. solani* (Mart.) Appel & Wollenw., *F. sporotrichioides* Sherb., and *F. equiseti* (Corda) Sacc. Although pathogenicity tests were not conducted to evaluate ability of isolates to elicit disease symptoms, it is expected both *F. proliferatum* and *F. oxysporum* are probably involved in causing disease symptoms exhibited by white pine transplants. Both species are common nursery pathogens (James and others 1991), with *F. proliferatum* very common on roots of container-grown conifers (Dumroese and others 1993) and *F. oxysporum* common on seed and within nursery soil (Bloomberg 1971; James 1986a, 1989a; James and others 1990). The other *Fusarium* spp. are probably less involved in disease (James and others 1991), although some *F. acuminatum* isolates may be aggressive pathogens (James and Gilligan 1984).

Pythium was the other group of fungi isolated from transplants. *Pythium ultimum* Trow. and *P. aphanidermatum* (Edson) Fitzpatrick were commonly isolated from chlorotic transplants, and less frequently from healthy transplants (Tables 3 and 4). Both species may be pathogenic to conifers in nurseries (Edmonds and Heather 1973; Hendrix and Campbell 1968), although *P. ultimum* is more frequently encountered (Husted 1988; James 1982). These fungi commonly reside in nursery soil and are less frequent in container operations which use soilless media (Chen and others 1987, 1988). Although they may be primary pathogens, especially under conditions of very high soil moisture or poor drainage (Bateman 1961; Griffen 1963; Kerr 1964), *Pythium* spp. also secondarily colonize roots damaged by other pathogens (Hendrix and Campbell 1973; Lifshitz and Hancock 1983).

Based on these results, it is suspected that much of the decline and mortality of plug+1 and plug+2 white pine transplants was due to extensive root colonization by several different plant pathogenic fungi which were mainly carried on plant roots from greenhouses to bareroot fields. Many of these fungi failed to elicit disease symptoms until the stress of storage (Blake 1983; Ritchie 1987) and subsequent transplanting (Rietveld 1989; Sands 1984; Stoneham and Thoday 1985) made infected plants vulnerable to damage. This, coupled with the lack of competitive organisms in soil due to fumigation, resulted in abnormally high disease. Evidence for secondary spread of root disease organisms was evident because of grouped concentrations of diseased transplants in several areas. Some of these concentrations were in noticeably low, poorly drained areas, which indicates increased activity by *Pythium* spp. (Bateman 1961; Griffin 1963). Root to root spread of some pathogenic fungi between diseased and non-diseased transplants may also have occurred.

An important question still unanswered is why some transplants became diseased and others did not. Levels of certain root-infecting fungi often do not significantly differ among plants displaying disease symptoms and those without such symptoms (James 1986b; James and Gilligan 1988a, 1988b; James and others 1994). Most cortical root tissues may be colonized with potentially pathogenic fungi when seedlings leave greenhouses (James 1986b; James and Gilligan 1988a). Of course, not all colonizing fungi are capable of causing disease under field conditions, even when host plants are severely stressed, because some isolates lack necessary pathogenicity genes (Bloomberg 1971, 1976; James and others 1991). However, fungal strains may be facultative parasites causing disease if host resistance is lowered or environmental conditions are especially conducive to disease development (Fisher and Toussoun 1983; James and others 1991). Unfortunately, isolates with different pathogenic potential are usually morphologically similar (James and others 1991; Nelson and others 1983). Therefore, the proportion of isolates obtained during this evaluation which were pathogens was unknown.

When seedlings with extensive fungal root colonization leave the nursery, they often appear healthy and perform well on forest sites after outplanting (Dumroese and others 1993; Smith 1967). *Fusarium* spp. often are replaced by other mycoflora once seedlings are outplanted (Smith 1967). Mycorrhizal fungi may also play an important role in limiting damage caused by *Fusarium* root infection (Sinclair and others 1975; Unestam and others 1987). However, if highly infected seedlings are transplanted into fumigated nursery soil, competition and antibiosis by soil organisms expected in "natural" soil will probably not occur. As a result, pathogens introduced on planting stock may exert greater damage than would normally be expected on forest sites. Buildup of pathogen levels may accelerate if natural biological controls are eliminated by soil fumigation (James 1989a). Such processes may have occurred in transplant beds severely damaged at the Coeur d'Alene Nursery.

The ideal way to prevent plug transplant damage would be to greatly reduce or eliminate levels of potentially pathogenic fungi on roots of container-grown seedlings. However, this may be impossible because of widespread occurrence of such fungi and ideal conditions for fungal spread and infection occurring in container operations (James and others 1987; 1991). Treating container stock with chemical fungicides after lifting may potentially help reduce levels of root pathogens, but has not been sufficiently tested. Limiting storage time between production in greenhouses and transplanting should help reduce transplant stress (Blake 1983; Ritchie 1987). Other possible methods to reduce damage would be mycorrhizal inoculation while seedlings are in greenhouses (Sinclair and others 1975) and keeping transplant areas non-fumigated (James 1989a). However, none of these methods have been sufficiently tested to evaluate efficacy in reducing transplant disease and mortality. Since transplant stress is undoubtedly an important contributing factor to

this problem (Rietveld 1989), any means of reducing stress should reduce future damage. Of course, growing seedlings in much larger containers to produce desired plant sizes may preclude need for transplanting. However, logistic and economic factors must be carefully evaluated in such a system.

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